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ORIGINAL COMMUNICATION

Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women

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Background: Although alcohol intake has been positively associated with breast cancer risk in epidemiologic studies, the mechanisms mediating this association are speculative.
Objective: The Postmenopausal Women's Alcohol Study was designed to explore the effects of moderate alcohol consumption on potential risk factors for breast cancer. In the present analysis, we evaluated the relationship of alcohol consumption with antioxidant nutrients and a biomarker of oxidative stress.
Design: Participants ($n = 53$) consumed a controlled diet plus each of three treatments (15 or 30 g alcohol/day or a no-alcohol placebo beverage), during three 8-week periods in random order. We measured the antioxidants, vitamin E (alpha (α)- and gamma (γ)-tocopherols), selenium, and vitamin C in fasting blood samples which were collected at the end of diet periods, treated and frozen for assay at the end of the study. We also measured 15-F_{2t}-IsoP isoprostane, produced by lipid peroxidation, which serves as an indicator of oxidative stress and may serve as a biomarker for conditions favorable to carcinogenesis.
Results: After adjusting for BMI (all models) and total serum cholesterol (tocopherol and isoprostane models) we observed a significant 4.6% decrease ($P = 0.02$) in α-tocopherol and a marginally significant 4.9% increase ($P = 0.07$) in isoprostane levels when women consumed 30 g alcohol/day ($P = 0.06$ and 0.05 for overall effect of alcohol on α-tocopherol and isoprostanes, respectively). The other antioxidants were not significantly modified by the alcohol treatment.
Conclusions: These results suggest that moderate alcohol consumption increases some biomarkers of oxidative stress in postmenopausal women.
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Introduction

The majority of epidemiologic studies support a positive relationship between alcohol consumption and breast cancer (Rosenberg *et al*, 1993; Longnecker, 1994), including a pooled analysis of six cohort studies by Smith-Warner *et al* (1998). Alcohol could influence breast cancer through several mechanisms, including altering the absorption and metabolism of protective antioxidant nutrients and increasing oxidative stress. Smoking, which generates oxygen free radicals and increases levels of lipid peroxidation products in blood, is associated with significantly lower blood levels of antioxidant nutrients after controlling for dietary intake

(Alberg, 2002). Antioxidants are thought to influence processes involved in carcinogenesis, including the prevention or repair of oxidative damage. Vitamin C, selenium, as well as α- and γ-tocopherol have all been associated with decreased risk for some cancers (Albanes & Hartman, 1999). Vitamin C traps free radicals and reactive oxygen molecules and selenium plays an important role in several proteins linked with tissue repair and cell-regulatory processes, including the repair and prevention of oxidative damage. Vitamin E is a free-radical scavenger and has been shown to inhibit lipid peroxidation (Albanes & Hartman, 1999). α-Tocopherol has higher bioavailability and concentration in the body, and has been studied more extensively than other forms of vitamin E; however, γ-tocopherol is the most abundant form in the US diet (Jiang *et al*, 2001).

There are three main pathways for ethanol metabolism in the hepatocyte: (1) the alcohol dehydrogenase pathway of the cytosol, (2) the microsomal ethanol oxidizing system in the endoplasmic reticulum, and (3) catalase located in the peroxisomes. All three pathways produce acetaldehyde, a highly toxic metabolite that promotes fatty acid synthesis and reduces lipid oxidation (Lieber, 2000). The microsomal ethanol-oxidizing system is induced by chronic alcohol consumption (Lieber, 1999). A key enzyme in this system, cytochrome *P*450 2E1 (CYP2E1), generates several species of active oxygen, and promotes lipid peroxidation (Novak & Woodcroft, 2000). High levels of reactive oxygen species can promote lipid peroxidation, damage cells, and contribute to chronic diseases, including cancer (Ames *et al*, 1993).

Morrow *et al* (1995) identified the F_2-isoprostanes, prostaglandin-like compounds produced by free radical-induced peroxidation of arachidonic acid that can be measured in plasma and urine. This group demonstrated that F_2-isoprostanes were superior to other biomarkers used as indices of lipid peroxidation *in vivo*, including malondialdeyde (MDA) (Longmire *et al*, 1994). Levels of F_2-isoprostanes in body fluids are elevated by conditions that are thought to be associated with free radical-induced oxidative stress, including smoking (Morrow *et al*, 1995; Reilly *et al*, 1996), hypercholesterolemia (Davi *et al*, 1997; Reilly *et al*, 1998; Palombo *et al*, 1999), diabetes (Davi *et al*, 1999), and acute and chronic alcoholic liver disease (Pratico *et al*, 1998; Meagher *et al*, 1999). Recent research suggests a role for oxidative stress in breast cancer (Kumar *et al*, 1991; Thangaraju *et al*, 1994; Li *et al*, 1999; Novak & Woodcroft, 2000; Ray *et al*, 2000).

The overall objective of the Women's Alcohol Study was to evaluate the effect of moderate alcohol consumption on potential risk factors for breast cancer. The effect of alcohol ingestion on serum hormones has been reported (Dorgan *et al*, 2001). A key secondary objective was to evaluate the effect of alcohol ingestion on antioxidant nutrients and other indicators of oxidative stress. In this manuscript, we report the results for the effects of alcohol on the concentration of α- and γ-tocopherol, α-tocopheryl quinone (an oxidation product of α-tocopherol), vitamin C, selenium, and 15-F_{2t}-IsoP isoprostane (formerly called 8-iso-$PGF_{2\alpha}$).

Materials and methods

Subjects

The Postmenopausal Women's Alcohol Study was conducted in 1998 and 1999 at the US Department of Agriculture's Beltsville Human Nutrition Research Center. Participants were recruited via advertisements from Beltsville, Maryland and surrounding communities. To be eligible, women had to be 50 y of age or older, nonsmokers, in good health, postmenopausal (last menses at least 1 y prior to entry), have at least one intact ovary, and not be using hormone replacement therapy or other prescription medication that might interfere with study endpoints. Further, women had to be willing to adhere to the study diet and could not be either an alcohol abstainer or have a personal or family history of alcohol abuse (themselves or their parents). All participants signed an informed consent before entering the study. A total of 57 women were enrolled into the study; four of these did not complete the study, leaving 53 for analysis. The study was approved by the institutional review boards at the National Cancer Institute, Bethesda, MD and the Johns Hopkins University Bloomberg School Public Health, Baltimore, MD.

Study design

Participants consumed a controlled diet plus each of three treatments (15 or 30 g/alcohol/day or a placebo beverage without alcohol), during three 8-week periods. The quantities of alcohol were chosen to approximate one and two average drinks per day. The treatments were provided in random order and separated by 2–5 week washout periods when women consumed no alcohol. All foods and beverages were prepared at the Beltsville Human Nutrition Research Center's Human Study Facility. Meals were prepared from typical foods using a 7-day menu cycle. On weekdays, participants consumed breakfast and supper at the Center and a carryout lunch was provided. All weekend foods and beverages were packaged for consumption at home with written instructions. Diets provided at least 100% of the recommended dietary allowances for vitamins and minerals. With the exception of calcium and iron that were prescribed by a physician, supplements were not allowed. The diets provided 15% energy as protein and 33% as fat, with a polyunsaturated:monounsaturated:saturated fat ratio of 0.6:1:1. The balance of dietary energy was from carbohydrate and alcohol. Daily cholesterol intake was 150 mg/1000 kcal and dietary fiber intake was 10 g/1000 kcal. Weight was monitored on each weekday and energy intake adjusted in 200 kcal increments as needed to maintain body weight throughout the study. Alcohol was supplied as 95% ethanol (Everclear) in 12 ounces of orange juice. Energy from alcohol was replaced with energy from carbohydrates

(Polycose™ and soft drinks) in the 0 and 15 g alcohol diets. Participants were asked to consume their study beverages with the snack provided by the investigators over a period 1–2 h before bed, after completing activities that require substantial manual dexterity (eg driving). Participants were not told the alcohol content of the beverages they received.

Laboratory methods

At the end of each treatment period, blood was collected in the early morning after an overnight fast. For the tocopherols, plasma (1 ml) was removed and added to 0.01 mg propyl gallate (an antioxidant preservative) prior to storage at −70°C. For selenium and vitamin C, serum was removed and protected from direct sunlight. Specimens for vitamin C analysis were treated with metaphosphoric acid prior to freezing at −70°C. For isoprostanes, plasma was removed, treated with BHT and frozen at −150°C. The tocopherols were extracted via an ethanol:hexane method and analyzed by a modification of the HPLC electrochemical detection technique described by Murphy and Kehrer (1987). Vitamin C (ascorbic acid) in serum was measured by isocratic high-performance liquid chromatography (HPLC) with electrochemical detection at 650 mV (Gunter *et al*, 1996). Selenium was measured in serum by graphite furnace atomic absorption spectrometry at 196.0 nm in a procedure based on the methods described by Paschal and Kimberly (1986). Measurement of 15-F_{2t}-IsoP isoprostane, was by a negative chemical ionization GC/MS method modified from that of Morrow and Roberts (1999; Parker *et al*, 2001). Samples for individual subjects were grouped in random order for analysis and were analyzed within the same batch. Masked quality control samples from a single pool were inserted randomly, but not between any individual subject's set of three samples. The coefficients of variation estimated from masked quality control samples overall were: α-tocopherol = 3%, γ-tocopherol = 10%, α-tocopheryl quinone = 22%, vitamin C = 27%, selenium = 10%, and isoprostanes = 13%. For vitamin C, although the overall CV was quite high, repeated quality control samples within the seven batches had CVs ranging between 0.8 and 7%. The tocopherols were analyzed in batches of 10; therefore, only one QC per batch was included making it impossible to evaluate CVs within batches for α-tocopheryl quinone, which also had a relatively high CV.

Statistical analysis

We used linear mixed models to test for differences in biomarker concentrations across treatment groups. Participant (subject) was treated as a random effect (ie a single random intercept) and alcohol levels as fixed effects designated by two indicator variables. These models allow for flexibility, testing for treatment effects as well as examining for period (time) and order effects (ie, testing for carry over effects), and adjusting for important baseline and time-dependent (ie serum cholesterol levels) covariates. Recently published manuscripts (Reilly *et al*, 1998; Palombo *et al*, 1999; Davi *et al*, 1997, 1999) demonstrated that serum cholesterol affects isoprostane levels, and previous research has suggested that blood tocopherol concentrations may be influenced by serum cholesterol levels. The alcohol treatments in this study significantly altered serum total cholesterol levels (Baer *et al*, 2002); therefore for vitamin E and isoprostane samples, we used a residual method to adjust concentration for the total serum cholesterol measurement obtained at the same time. Residuals were generated from regression models with either plasma isoprostane or tocopherol as the response variable and serum total cholesterol as the explanatory variable. The mean plasma isoprostane/tocopherol concentration was then added to the residual to generate a meaningful value for cholesterol-adjusted concentration. Antioxidant and isoprostane concentrations were analyzed as untransformed and transformed to the $\log_e$. We had similar results for transformed and untransformed variables concentrations; therefore, we report the results for the untransformed data. We evaluated the effects of other variables including age, body mass index (BMI = weight (kg)/height (m^2)), race, nutrient status at baseline, serum low density lipoprotein (LDL) and high density lipoprotein (HDL) status, treatment period and treatment order for contribution to overall fit or improvement in precision of the model. Likelihood ratio tests were used to evaluate whether covariates improved model fit and also to test for treatment effect. Treatment effect was tested by simultaneously testing whether the fixed effects covariates corresponding to the two treatment effect indicators were zero (χ^2 with 2 degrees of freedom). We also tested whether each treatment level (15 and 30 g alcohol/day) was significantly different from the no alcohol level by individually testing whether these two coefficients were zero (χ^2 with 1 degree of freedom for each test). Standard errors of alcohol estimates from simple models and models that included characteristics significantly associated with isoprostane/antioxidant concentration were compared to evaluate the effect of adjustment on precision. We adjusted for BMI in our analyses since BMI was a significant predictor and resulted in a more precise estimate of alcohol effects. In addition, others (Dietrich *et al*, 2002) have reported that BMI is correlated with F_2-isoprostane level. Other covariates such as age, race, baseline nutrient status, period, and treatment order had no effect on our inferences for alcohol. Effect modification by treatment order, body mass index, and age was assessed by likelihood ratio tests of improvement in model fit after addition of the interaction (cross product) terms to models that included the main effects for alcohol (two indicator variables) and the characteristic of interest. All analyses were performed using SAS (SAS/Stat version 6, SAS Institute, Cary, NC, USA). A *P*-value of 0.05 was considered statistically significant.

Results

Baseline characteristics of the study participants are shown in Table 1. Mean age for the group at baseline was approximately 60 y and mean BMI was 27.8 kg/m^2. The majority of the participants were white (73%), 23% were African American, and 4% were Asian. Mean serum cholesterol concentrations were 208 at baseline (before the controlled diet) and 213, 208, and 208 mg/dl when participants consumed no alcohol, 15 g alcohol, and 30 g alcohol/day, respectively.

During the diet treatment periods, the average calculated dietary intake of vitamin C for the 1800 and 2200 kcal diets was estimated at 148 and 181 mg/day, respectively, well above the recommended 75 mg/day for women in this age group (National Academy of Sciences, 2000). We analyzed weekly composite samples of the 1800 and 2200 kcal study diets for tocopherols and selenium to estimate daily intake of these nutrients. Average daily intake of tocopherols on the 1800 and 2200 kcal diets was 11.4 and 12.3 mg for α-tocopherol, 31.4 and 39.6 mg for γ-tocopherol, and 13.3 and 15.4 mg for δ-tocopherol. α-Tocopherol equivalents (ATeq) were defined as follows: α-tocopherol, mg $\times 1.0$; γ-tocopherol, mg $\times 0.1$; δ-tocopherol, mg $\times 0.03$. Average total vitamin E intake as ATeq was 15.0 and 16.7 mg/day for the 1800 and 2200 kcal diets, respectively. The recommended intake for vitamin E is 15 mg/day ATeq (National Academy of Sciences, 2000). Selenium intake averaged 114 μg/day on the 1800 kcal and 131 μg/day on the 2200 kcal diets, approximately two times the recommended intake for women ages 51 and older of 55 μg/day (National Academy of Sciences, 2000).

Table 2 shows participants' mean antioxidant and isoprostane concentrations after consumption of the placebo (no alcohol) and mean change in concentration for the two alcohol treatments after adjusting for covariates. The overall *P*-value for the effect of alcohol on α-tocopherol concentration in the model was 0.06 and the *P*-value for the effect on isoprostane concentration was 0.05. α-Tocopherol concentration decreased 4.6% ($P=0.02$) and isoprostane concentration increased 4.9% ($P=0.07$) on the 30 g alcohol/day treatment. The pattern of change observed for these two biomarkers was different. For example, the concentration of serum α-tocopherol was decreased, although not significantly, on the 15 g/alcohol/day treatment, while increase in

Table 1 Participant characteristics at baseline ($n=53$)

	Mean	*95% confidence interval*
Age (y)	59.7	57.7, 61.9
Height (cm)	163.7	162.0, 165.4
Weight (kg)	74.4	69.9, 78.9
Body mass index (kg/m^2)	27.8	26.6, 29.4
Serum cholesterol (mg/dl)	207.8	198.6, 217.0
Usual no. alcoholic (beverages/week)	0.9	0.2, 1.5
	Number	*Percent*
Race		
White	39	73
African American	12	23
Asian American	2	4
Education[a]		
<High school	5	9
High school graduate	18	34
College/graduate work	28	53

[a]Responses for two individuals were missing.

Table 2 Mean antioxidant and F_2-isoprostane levels and 95% confidence intervals when consuming no alcohol and change when consuming 15 and 30 g alcohol/day[a]

	No alcohol	*15 g/day*[b]	*30 g/day*[b]	*Percent change (0 vs. 30 g)*	*Overall alcohol* P-*value*
α-Tocopherol (μM)	26.3 (24.6,28.1)	25.5 (23.8,27.3)	25.1 (23.3,26.9)	−4.6	0.06
Difference		−0.8 (−1.8,0.2)	−1.2 (−2.2,−0.2)		
		$P=0.12$	$P=0.02$		
α-Tocopheryl Quinone (μM)	0.071 (0.07,0.08)	0.070 (0.06,0.07)	0.072 (0.07,0.08)	−0.1	0.39
Difference		−0.002 (−0.006,0.002)	0.001 (−0.003,0.005)		
		$P=0.38$	$P=0.64$		
γ-Tocopherol (μM)	4.0 (3.6,4.4)	3.9 (3.5,4.3)	4.1 (3.7,4.5)	2.5	0.07
Difference		−0.1 (−0.3,0.1)	−0.1 (−0.1,0.3)		
		$P=0.17$	$P=0.37$		
Selenium (ng/ml)	130 (127,134)	131 (127,135)	132 (128,136)	1.5	0.56
Difference		0.7 (−2.1,3.5)	1.5 (−1.3,4.3)		
		$P=0.63$	$P=0.29$		
Vitamin C (mg/dl)	0.682 (0.610,0.754)	0.670 (0.598,0.743)	0.663 (0.591,0.735)	−2.3	0.82
Difference		−0.012 (−0.072,0.049)	−0.019 (−0.080,0.041)		
		$P=0.70$	$P=0.53$		
Isoprostanes (ng/ml)	0.264 (0.245,0.282)	0.260 (0.242,0.278)	0.277 (0.259,0.295)	4.9	0.05
Difference		−0.003 (−0.017,0.010)	0.013 (−0.001,0.027)		
		$P=0.63$	$P=0.07$		

[a]Model adjusted for BMI for all variables and also for total serum cholesterol level for vitamin E and isoprostanes.
[b]These differences and *P*-values are for the respective comparisons with the no alcohol treatment.

isoprostane concentration was apparent only at the higher level of alcohol intake. The mean isoprostane concentration on the 30 g alcohol treatment was significantly greater than the mean isoprotane concentration for the no alcohol and 15 g alcohol treatments combined (adjusted means of 0.277 and 0.262 ng/ml, respectively; $P = 0.02$). We saw no statistically significant changes in α-tocopherol quinone, γ-tocopherol, vitamin C, and selenium concentrations when women consumed either 15 or 30 g/alcohol/day.

The effect of alcohol on antioxidant or isoprostane concentrations did not vary by age, BMI, serum cholesterol concentration, diet period, or ordering of the treatments.

Discussion

In this study, we observed an increase of approximately 5% in mean plasma isoprostane concentration and a decrease −4.6% in plasma α-tocopherol concentration when postmenopausal women consumed the equivalent of two alcoholic beverages/day (30 g/day) during an 8-week controlled feeding period. To our knowledge, this is the first study to suggest that chronic consumption of moderate amounts of alcohol by healthy postmenopausal women may lead to significant changes in biomarkers associated with oxidative stress.

Cellular oxidative damage is a well-established mechanism for tissue injury, and has been suggested as a factor in many disease processes, including carcinogenesis (Ames *et al*, 1993). Oxidative stress occurs when the formation of active oxygen metabolites exceeds the scavenging of these substances by antioxidants. In cell membranes, polyunsaturated fatty acids, one of the prime targets of reactive oxygen species, may undergo lipid peroxidation, leading to damage of the cell structure and function (Ames *et al*, 1993).

Oxidative stress may play an important role in breast cancer, and could be a common mechanism through which sex hormones, dietary intake, and exposure to carcinogens interact to promote cancer development. In a study of 87 women with breast cancer and 29 control women, Li *et al* (1999) found that cancer patients had significantly higher levels of lipid peroxidation-related DNA adducts in breast tissue samples than controls. Ray *et al* (2000) evaluated malondialdehyde (MDA) concentration, an index of lipid peroxidation, along with activity of antioxidant enzymes in 54 breast cancer patients and 42 age-matched controls. Their results showed that irrespective of menopausal status, MDA concentration was significantly elevated in patients with stage II or greater breast cancer. In a study of 25 women with breast cancer, 25 with fibrocystic breast disease, and 19 healthy controls, Afrasyap *et al* (1998) observed increases in antioxidant enzyme activity in plasma and erythrocytes among patients with breast cancer. Unfortunately lipid peroxidation was not measured in their study. Huang *et al* (1999) found that serum MDA concentrations were significantly elevated in 35 breast cancer patients compared to 35 controls. These investigators also noted that levels of selenium were significantly lower and copper significantly higher in patients with breast cancer. Similarly, Kumar *et al* (1991) reported that lipid peroxidation was increased and levels of the antioxidant nutrients, selenium and vitamins C and E, were decreased in women with breast cancer compared to age-matched controls. In addition, women with more advanced breast cancer had more lipid peroxides than women with localized cancers. Lastly, the prescription drug Tamoxifen, used widely as an antiestrogen in the treatment of breast cancer, has been shown to reduce lipid peroxidation and decrease serum MDA levels in breast cancer patients (Thangaraju *et al*, 1994).

There are some limited data from other studies that suggest alcohol and antioxidant nutrients play a role in lipid peroxidation. Colantoni *et al* (2000) demonstrated that after 8 weeks, rats fed a diet that contained 36% of their total energy intake as alcohol had increased MDA levels. Kawse *et al* (1989) measured hepatic and plasma lipid peroxidation and vitamin E in alcohol-fed rats. The hepatic content of α-tocopherol, α-tocopheryl quinone and the ratio of α-tocopherol/plasma lipid was reduced by ethanol feeding; however, hepatic vitamin C levels increased. Plasma levels of vitamin C and α-tocopheryl quinone were not reported in this study. In another animal study, Eskelson *et al* (1993) reported that mice consuming higher levels of vitamin E were protected from alcohol-induced free radical activity, and the incidence of chemically induced cancers decreased, in vitamin E-supplemented animals compared to controls. Similar results were reported by Jordao *et al* (2004) in a recent animal study that measured both plasma and liver lipid peroxidation after an acute dose of ethanol administered to 120 male rats fed three levels of vitamin E (deficient, control, supplemented). Prior to the alcohol treatment, liver lipid peroxidation levels were low in control and vitamin E-supplemented animals compared to the deficient group. Administration of ethanol lowered mean hepatic vitamin E concentration in all groups and significantly increased lipid peroxidation among the control and vitamin E-supplemented groups. The authors concluded that alcohol increased lipid peroxidation both by increasing levels of reactive oxygen species and via a decrease in antioxidant status. Meagher *et al* (1999) evaluated the effects of an acute dose of alcohol on urinary isoprostane levels in subjects ($n = 5$ male, 5 female subjects) who received 0.2–0.9 g/kg body weight of alcohol in a lemonade solution. Blood alcohol and urinary isoprostane levels were evaluated at 20, 40 and 60 min and at 2, 3, 4, 6, 12, and 12 h after dosing. Results indicated that alcohol significantly increased peak urinary isoprostane excretion in a dose-dependent manner approximately 6 h after dosing. Sodergren *et al* (1999) recently reported that rats receiving 3 weeks of vitamin E supplementation had decreased total levels of urinary F_2-isoprostanes and free F_2-isoprostanes in liver. These investigators observed no differences in plasma isoprostane concentrations between rats supplemented with vitamin E and control rats. They

hypothesized that urinary isoprostane levels reflect an earlier event in the biosynthesis and availability of these compounds compared to plasma levels measured at the same time. Lastly, Djuric *et al* (1998) reported that oxidative DNA damage levels (5-hydroxymethyluracil) in blood from women at high risk for breast cancer were negatively associated with intake of antioxidant-rich vegetables and fruits and positively associated with consumption of red meats.

The results from some controlled studies conducted with cigarette smokers suggest that measurable changes can occur in the antioxidant defense system over a short time period. Reilly *et al* (1996) demonstrated that among chronic cigarette smokers, urinary concentrations of 8-iso-PGF2α (15-F_{2t}-IsoP isoprostane) were reduced after 5 days of supplemental vitamin C (2 g) or vitamin C and vitamin E (800 IU) together, but not supplemental vitamin E alone. Steinberg and Chait (1998) observed that daily supplementation with 600 mg of vitamin C, 400 mg of vitamin E and 30 mg of β-carotene for 8 weeks reduced lipid peroxidation among hyperlipidemic cigarette smokers. Similarly, in a group of 15 healthy men and women who were followed for 4 weeks after smoking cessation, crude levels of plasma vitamin C increased by 26%, vitamin E increased by 11% and malondialdehyde (MDA) decreased by 28% (Polidori *et al*, 2003). In this study, diet was not controlled, but a food frequency questionnaire was administered pre- and post-cessation and suggested that diet did not change significantly. In the largest randomized controlled trial to date of nonsmokers ($n = 184$), Huang *et al* (2002) evaluated the main and synergistic effects of supplementation with vitamin C (500 mg/day) and E (400 IU/day) on urinary F2-isoprostanes. After 2 months, urinary isoprostane concentration decreased by approximately 10% with either supplement and no further reduction was seen when the two supplements were consumed together.

In the current study, we did not see significant changes in α-tocopheryl quinone, γ -tocopherol, vitamin C or selenium status. In interpreting the results of our study, it is reasonable to question why we observed significant changes to some of the biomarkers measured and not others. Jiang *et al* (2001), in a recent review, suggest that the metabolism of the two isomers of vitamin E measured in this study is quite different, thus γ-tocopherol may not have changed due to the alcohol treatment despite the observed reduction in α-tocopherol levels. Other research has demonstrated that α-tocopherol is consumed more quickly in oxidative reactions than γ-tocopherol (Ma *et al*, 1994). In contrast to α-tocopherol (CV 4%), γ-tocopherol and α-tocopheryl quinone were measured with less precision (CVs 10 and 22%, respectively); thus, it is also possible that levels may have changed due to alcohol consumption, yet we were unable to detect significant differences. We evaluated the dietary intake of the antioxidants measured in blood in this study. The mean vitamin C intake during this controlled feeding study approached three times the Dietary Reference Intake (DRI), mean selenium intake approached two times the DRI, and mean vitamin E intake during the study was approximately equal to the DRI. Therefore, it is possible that higher dietary intakes of vitamin C and selenium compensated for any modest effects due to the alcohol treatment.

We measured selected antioxidant nutrients and one marker of oxidative stress in blood, which may or may not reflect what is happening at the tissue level. However, blood is often used for the evaluation of free radical-induced damage. Methods based on the characterization of serum or plasma parameters are considered representative of the antioxidant status of the whole organism because they reflect the integrated antioxidant status which arrives from both body tissues and nutrition (Chevion & Chevion, 2000). Lastly, we observed a 5% increase in plasma isoprostane levels when women consumed 30 g/day of alcohol. In this study, blood was drawn approximately 12 h after alcohol ingestion. If the effect of alcohol on isoprostanes peaks within 6 h, as noted by others (Meagher *et al*, 1999), we may actually have underestimated the effect of alcohol on isoprostane levels.

It is unknown whether a 5% increase in oxidative stress or similar decrease in α-tocopherol represents a biologically meaningful change. These changes were observed over a relatively short time frame among healthy women who were consuming a diet adequate in all nutrients. Presumably, the effects of many years of higher levels of alcohol intake among women with poor diets would be greater. A comparison of our subjects' baseline dietary intakes to those during the study suggests that the study diet provided more antioxidants and other essential nutrients than their free-living baseline diets. Prolonged fasting decreases ethanol oxidation rates, and at a given alcohol intake, poorly nourished individuals develop higher blood alcohol levels and sustain them longer than well-nourished individuals (Lieber, 2000).

In summary, we observed a decrease in plasma α-tocopherol and an increase in plasma isoprostane concentrations with chronic moderate alcohol consumption in postmenopausal women. Alcohol has numerous physiological effects; however, this may be one means through which alcohol consumption increases risk for breast cancer in postmenopausal women.

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